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Effect of the secretory leucocyte proteinase inhibitor (SLPI) on *Candida albicans* biological processes: A therapeutic alternative?

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of SLPI on the growth and biological processes of *Candida albicans*.

Methods: Two *C. albicans* strains were used in this study, a clinical isolate resistant to fluconazole (PRI) and a reference strain ATCC 24433. The minimal inhibitory concentration (MIC) was determined according to the CLSI methodology. The influence of SLPI on secreted serine proteinase activities (SSP) was measured by the cleavage of specific substrate, and surface hydrophobicity was determined by the aqueous-hydrocarbon biphasic separation method. Flow cytometry was performed to investigate receptors for SLPI and variations in the cell wall mannoprotein expression. Interaction between yeast and epithelium was assessed using the MA-104 cells lineage. Ultrastructure was analyzed by transmission electron microscopy (TEM).

Results: MIC values were calculated as 18 and 18.9 μM for the PRI and ATCC 24433, respectively. SSP activity was reduced by 48.8% by 18 μM of SLPI and cell surface hydrophobicity increased by 11.1%. Flow cytometry suggest the existence of SLPI binding sites on the surface of the yeast. Results showed a reduction in the expression of mannoproteins in 20.8% by the cells treated with 80 μM of SLPI, and 18 μM reduced the adhesion of yeasts to mammalian cells in 60.1%. TEM revealed ultrastructural changes in cells treated with 80 μM of SLPI, such as the presence of membrane-like structures within the cytoplasm.

Conclusions: SLPI exerts a significant influence on *C. albicans* viability and biological processes. Considering its constitutive and physiologic features, SLPI may become a promising tool for the development of new methodologies for the treatment and control of candidiasis.

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1. Introduction

Candidiasis is a major opportunistic fungal infection in humans and its incidence has increased steadily over the last two decades due to the increasing number of susceptible individuals, such as transplant patients and HIV + individuals,[1] as well as the population ageing and the extensive use of aggressive antimicrobial and chemotherapeutic agents.[2–4] This situation is further aggravated due to the limited number of antifungal drugs commercially available, that does not allow an efficient control of the infection caused by this fungi.[5] For many years, fungal infections were treated with polyene compounds (Nystatin and Amphotericin B), despite their high toxicity. In the early 90s, the development of first generation triazoles (fluconazole and itraconazole) changed the epidemiology of candidiasis by offering new options for prevention and treatment.[6] However, the extensive use of these new antifungal agents has resulted in resistant strains, which now creates urgency for the development of new treatment strategies.[7,8]

Human candidiasis infections can range from a mild disease to a severe systemic infection that can be fatal.[9] Such variations occur, in large part, due to virulence factors that can determine the course of the infection. In candidiasis, these factors are responsible for the success of the colonization or infection in the host. *Candida albicans*, in particular, has developed an effective set of virulence attributes and specific strategies that can determine the course of infection.[10,11] Although there are many virulence factors that have been attributed to *C. albicans*, hydrophobicity; adherence to the host tissues; morphological conversion to the filamentous form (dimorphism); recognition of surface molecules and enzymes production are considered the most important issues to date.[12–14] Therefore, considering the role of extracellular enzymes in the pathogenesis of candidiasis, the therapeutic use of protease inhibitors could act to reduce the virulence of *C. albicans*.[15]

The secreted leucocyte protease inhibitor (SLPI) is a cationic, non glycosylated protein, consisting of 107 amino acids with a low molecular weight (11.7 kDa),[16–19] able to inhibit the activity of a large number of serine proteases.[17,20,21] SLPI can be found in several mucosal secretions, such as saliva, milk and semen, in different concentrations, ranging from 2.0 μM in saliva[22–25] to 80 μM in seminal fluid.[26] The therapeutic potential of SLPI was demonstrated "in vitro" when applied on induced epidermal lesions, which resulted in the reduction of the lesion size, the tissue repair time and inflammation.[27,28] In addition, SLPI antimicrobial features have already been reported.[29–32] Therefore, the enzymatic inhibitory features of this protein associated to its antifungal activity suggest its potential for the development of new therapeutic strategies against candidiasis. Considering this, this study aimed to evaluate the antifungal potential of SLPI on *C. albicans* biological processes, which may contributes to the development of new strategies for therapeutics and prophylaxis of candidiasis.

This study shows for the first time the ability of SLPI to modulate different virulence factors, such as: the proteolytic activity, hydrophobicity, adhesion to epithelium and mannoprotein expression on *C. albicans*. These factors were particularly selected due to its essential role in the very early stages of the infections, providing the installation of the disease. In addition, the ultrastructural changes that this protein causes on the yeast were evaluated by TEM.

2. Materials and methods

2.1. Strains and culture conditions

Despite the emerging shift in the aetiology of candidiasis, *C. albicans* remains the most prevalent species in mucosal infections,[33–36] skin infections[37] and in nosocomial candidemia.[38] Thus, two strains of *C. albicans* were chosen to be evaluated in this work. One clinical strain resistant to fluconazole, which was isolated from the oral mucosal of an HIV(+) patient, namely PRI,[39,40] that was used in all experiments, and the ATCC 24433 reference strain, which was used in the MIC and in the minimal fungicidal concentration (MFC) assays. Before any experimental procedures, both strains were subcultured into Brain Heart Infusion (BHI) broth and incubated at 37 °C for 48 h for the yeasts to reach the exponential growing phase.

2.2. Recombinant protein

Recombinant SLPI protein and anti-hSLPI antibody were purchased from R&D System® (Minneapolis, USA) and reconstituted in phosphate-buffered saline PBS 10 mM, pH 7.2 at 25 °C.

2.3. MIC and MFC

SLPI MIC was determined according to the M27-A2 methodology for microdilution. Serial protein dilutions were prepared in the range from 80 to 0.62 μM, and fluconazole and nystatin were used as control. Microtitre plates were evaluated spectrophotometrically (600 nm), and the results were analyzed through the linear equation obtained by dispersion analysis in Microsoft Excel®. The minimum concentration able to inhibit yeast growth completely and 50% were defined as MIC and IC_{50}, respectively. MFC was calculated by subculturing 10 μL of each well onto Sabouraud dextrose agar (SDA) medium in Petri dishes, and incubated at 37 °C for 24 h. The lowest protein concentration able to decrease yeast viability by 99.9% was considered to be MFC.

2.4. Proteolytic activity

The inhibitory function of SLPI towards the proteolytic activity of secreted serine proteinases from the *C. albicans* clinical strain was evaluated by the cleavage of the fluorogenic substrate Z-Phe-Arg-4-methoxy-β-naphthylamide (Sigma Aldrich®, St. Louis, USA). After a 48 h incubation in BHI medium, yeast cells were harvested by centrifugation for 8 min at 4 °C, the supernatant was filtered in a Millipore® (Billerica, MA, USA) membrane 0.22 μm and concentrated by ultrafiltration with an exclusion membrane of 10 kDa, for 7 h at 4 °C resulting in a 30× concentrated solution.[8] The

concentrated supernatant (20 μL) was diluted in 50 μL of a reaction buffer containing sodium acetate 0.1 M, pH 5.0 and 2.5 μL of the fluorogenic substrate Z-Phe-Arg-4-methoxy-naphthylamide and incubated at 37 °C for 1 h with different concentrations of SLPI (2.0 and 18 μM) that were added during the hydrolysis process. The reaction was stopped by the addition of 150 μL of trichloroacetic acid 5%. Substrate cleavage was fluorometrically read at wavelengths of 380 and 450 nm for fluorescence excitation and emission, respectively.[41]

2.5. Cell surface hydrophobicity (CSH)

CSH was determined by the aqueous-hydrocarbon biphasic hydrophobicity assay. Briefly, 1×10^7 cells were incubated in 80 μM of SLPI (highest physiologic concentration) for 6 h at 37 °C, washed in cold deionized water and suspended in phosphate urea magnesium (PUM) buffer, pH 7.1, and optical density was adjusted to 0.400 at 600 nm. Yeast samples (1.2 mL) were added to 300 μL of cyclohexane in borosilicate glass tubes and vigorously vortexed for 3 min. After decanting, the aqueous portion was measured at the same wavelength. The percentage difference between readings of the aqueous phases before and after cyclohexane addition was considered to be the hydrophobicity value of the cell population.[42]

2.6. SLPI binding to C. albicans wall

Flow cytometry analysis was carried out in order to investigate ligants for SLPI on the surface of the *C. albicans* clinical strain. Briefly, 10^6 cells were washed in PBS 10 mM, pH 7.2, fixed in paraformaldehyde 4%, blocked with bovine serum albumin (BSA) 5%, and incubated with 2 (lowest physiological concentration) or 9 μM of SLPI for 90 min at 25 °C. Cells were then washed and incubated with 10 mg/L of SLPI antibody (anti-hSLPI Affinity Purified Goat IgG, R&D System® Minneapolis, USA) for 1 h, and incubated with secondary antibody anti goat IgG conjugated to fluorescein isothiocyanate (FITC) (Sigma Aldrich® St. Louis, USA), in a 1:1000 dilution. Yeast cells (10 000 events) were evaluated in a flow cytometer EPICS ELITE (Coulter Electronics, Hialeah, FL, USA) equipped with 15 mW argon laser at a wavelength of 488 nm, and results were analyzed using the WinMDI software version 2.8. Control cells (cells that were only washed and fixed) were used to determine autofluorescence.

2.7. Cell wall mannoprotein expression

In order to evaluate variations in the presence or expression of cell wall mannoproteins, flow cytometry analysis was carried out. Yeasts cells (1×10^7) were incubated with 9.0 (IC_{50}) and 80 μM of SLPI for 6 h at 37 °C. After incubation, cells were washed in PBS 10 mM, pH 7.2, fixed in paraformaldehyde 4%, blocked with BSA 5%, and incubated with FITC labelled Concanavalin-A (Sigma Aldrich® St. Louis, USA), (2 mg/ml) in a 1:500 dilution. Yeasts were washed and 10 000 events were acquired for each analysis in an EPICS ELITE® flow cytometer (Coulter Electronics, Hialeah, FL, USA) equipped with a 15 mW argon laser at a wavelength of 488 nm.

2.8. Adhesion of C. albicans to epithelial cell

Epithelial cells from monkey kidneys (MA 104) were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), and 1×10^5 cells were placed onto glass coverslips in a 24 well culture plate which was incubated at 37 °C in a 5% CO_2 humidified atmosphere for 24 h. Yeast cells were incubated with the mammal cells at a 10:1 ratio in DMEM with or without 18 μM (MIC) of SLPI for 90 min at 37 °C. Un-adhered yeasts were removed by extensive washes with PBS 10 mM, 7.2 pH, and coverslips were fixed with Bouin, stained with Giemsa, dehydrated in acetone and xylol and then mounted on Permount® (Fisher Chemicals, Leicestershire, UK). The number of associated yeasts per 100 epithelial cells was estimated by counting under optical microscopy at a 1000× magnification with an Axioplan 2® microscope (Zeiss, Jena, Germany).[12]

2.9. Ultrastructure

To evaluate possible ultrastructural changes caused by SLPI, the yeasts were treated and analyzed by transmission electronic microscopy (TEM). The cells (1×10^7) were incubated with 9.0 and 80 μM of SLPI for 6 h at 37 °C, washed in PBS 10 mM, pH 7.2 and fixed in 2.5% glutaraldehyde in sodium cacodylate buffer 0.1 M, pH 7.2 for 1 h. Fixed samples were washed and post-fixed in 1% osmium tetroxide in sodium cacodylate buffer 0.1 M, pH 7.2 containing 0.8% potassium ferrocyanide and 5 mM of $CaCl_2$. Dehydration was carried out by increasing concentrations of acetone, and samples were embedded in epoxy resin to make the blocks which were polymerized at 60 °C for 72 h. Blocks were cut on an ultramicrotome and sections were placed on copper grids and contrasted in saturated uranyl acetate and lead citrate solution. Images were finally analyzed in a transmission electron microscope EM 900® (Zeiss, Oberkochen, Germany).

2.10. Statistical analyses

Flow cytometry final results were considered to be the representation of four independent experiments. All others experiments were performed at least in triplicate and results were expressed as mean ± standard deviation. Data were analyzed by the Student's *t*-test paired, and p values lower than 0.05 were considered significant.

3. Results

3.1. MIC and MFC

The MIC data of the clinical isolate and ATCC strain of *C. albicans* are summarized in Table 1. The results show that SLPI was able to inhibit yeast growth in both strains in similar concentrations. Equivalence between molarity and weight/volume concentrations was registered as well, and MIC and IC_{50} were equivalent to 18 and 18.9 and 9.9 and 9.4 μM for the clinical isolate and ATCC 24433 strains, respectively. MFC for both strains were calculated as 20 μM, suggesting primarily a fungicidal activity.

Table 1 – MIC and IC_{50} of SLPI, fluconazole and nystatin assessed on *C. albicans* ATCC 24433 and clinical isolate, according to the CLSI M-27A2 protocol. Cell growth was measured spectrophotometrically (600 nm).

C. albicans	SLPI (μM)		Fluconazole (mg/L)		Nystatin (mg/L)	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
Clinical isolate	18 ± 2.6	9.9 ± 1.2	>128	>128	9.67 ± 0.5	4.06 ± 0.7
ATCC 24433	18.9 ± 0.5	9.4 ± 0.2	2.81 ± 2.7	1.51 ± 1.8	nt	nt

nt = not tested.

3.2. Proteolytic activity

The proteolytic activity profile of secreted serine proteases released by the clinical isolate in the presence of different SLPI concentrations are summarized in Fig. 1. The figure shows that the minimal physiologic concentration (2.0 μM) and MIC (18 μM) of SLPI were able to inhibit the proteolytic activity of serine protease secreted by *C. albicans* in 37.1% and 48.4%, respectively.

3.3. Hydrophobicity

Cell surface hydrophobicity was determined by the aqueous-hydrocarbon biphasic partitioning assay and revealed that when the yeasts were incubated with the highest physiologic concentration (80 μM) of SLPI for 6 h at 37 °C, CSH reached 27.2%, whereas CSH was 16.1% in the incubation cells without SLPI, indicating an increase of 11.1% in the yeast CHS after SLPI treatment.

3.4. Surface receptors for SLPI

The histogram presented in Fig. 2 demonstrates that SLPI was able to recognize specific molecules on the surface of *C. albicans*. Fluorescence peaks moved to the right when yeast cells were treated with the lowest physiological concentration of SLPI 2 μM (Fig. 2A) and 9 μM of SLPI (Fig. 2B). However this latter incubation shows a greater peak displacement, compared to the first one, pointing to a higher level of recognition. For confirmation, arbitrary fluorescent units (AFU) were measured, and they showed an increase in fluorescence emission of 21.1% and 70% when the yeasts were incubated with 2 and 9 μM of SLPI, respectively.

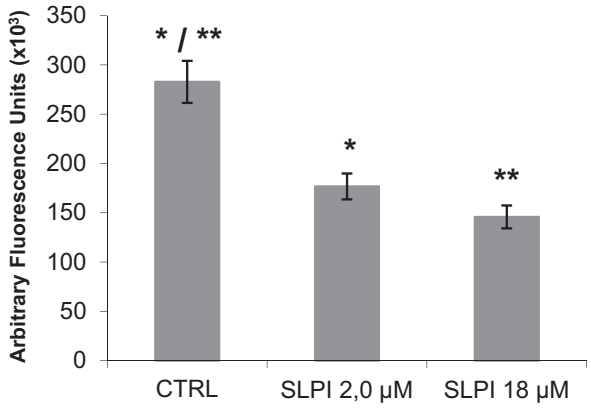

Fig. 1 – Effect of SLPI on proteolytic activity of serine proteases secreted by *C. albicans*. The concentrated supernatant was diluted with buffer containing the fluorogenic substrate in the absence (CTRL) or presence of 2.0 and 18 μM SLPI. The percentage difference between the fluorometric readings was considered the inhibition of proteolytic activity $p < 0.001$ (*); $p = 0.001$ (**).

3.5. Cell wall mannoprotein expression

Flow cytometry analysis using concanavalin-A (con-A) as a probe for mannoproteins revealed that treatment with the IC_{50} of SLPI (9.0 μM) did not alter the expression of receptors for the lectin on the surface of *C. albicans* (Fig. 3). However, when cells were incubated with the highest physiologic concentration (80 μM) of SLPI, the flow cytometry analysis revealed a decrease in the expression of mannoproteins on the cell wall of the yeast. Considering only AFUs, there was a decrease of 20.8% in the fluorescence emitted by yeast cells treated with 80 μM of SLPI, while no shrinkage was observed in the 9.0 μM incubation.

3.6. Adhesion of *C. albicans* to epithelial cells

The influence of 18 μM of SLPI on the interaction between the clinical strain and MA 104 is demonstrated in Fig. 4. SLPI was able to reduce the association index by 60.1%, as shown in Fig. 4(a). Representative images of the interaction process are shown in Fig. 4(b), where a clear distinction between the interaction patterns can be seen, strengthening the results of quantitative analysis.

3.7. Ultrastructure

Transmission electron microscopy revealed ultrastructural changes in cells treated with 9.0 (data not shown) and 80 μM of SLPI (Fig. 5). Treatment with 80 μM SLPI caused cell deflation and structural changes such as the presence of disorganized membrane structures within the cytoplasmatic compartment.

4. Discussion

Growth inhibition can contribute substantially to control fungal infections, since the ability to limit the proliferation of rapidly growing fungi can decrease the onset of the disease in susceptible patients.[43] Fluconazole displays linear pharmacokinetics in dosages that can reach 50–800 mg/day, it has a high bioavailability, and low binding to circulating proteins, which provides an easy and quick access to patient's tissues.[3] Cutoff points that determine the resistance or susceptibility of *C. albicans* to fluconazole are well established and MIC values

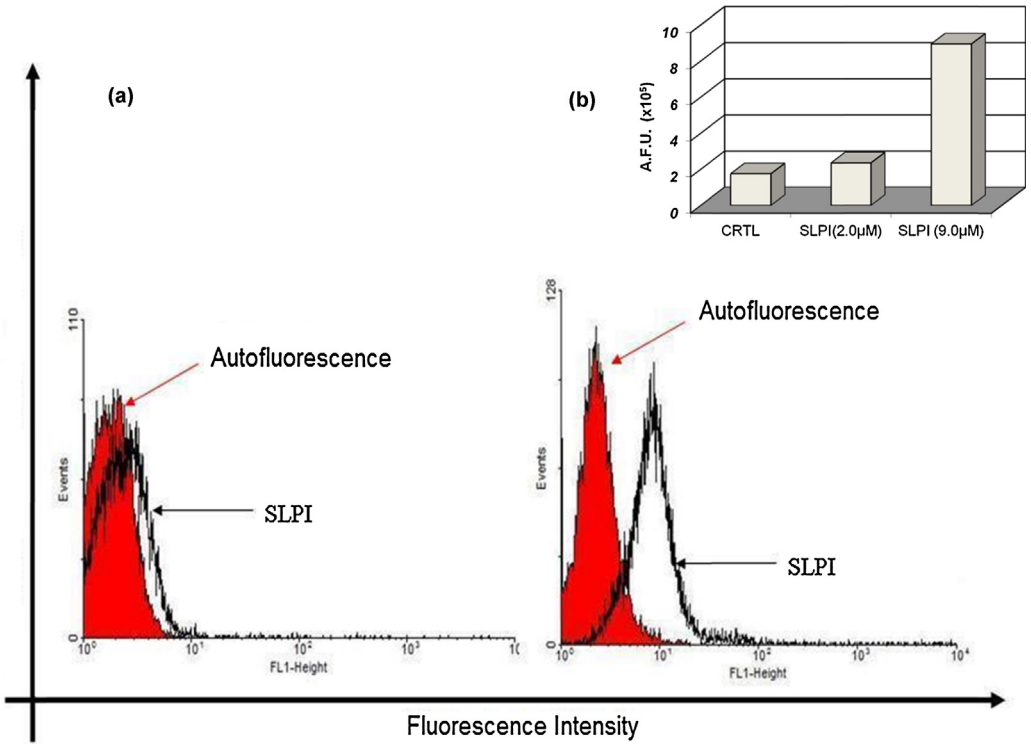

Fig. 2 – Analysis by flow cytometry showing binding of SLPI to the surface of C. *albicans*. Yeasts were incubated with 2.0 μM SLPI (a), or 9.0 μM of SLPI (b) for 90 min, followed by incubation with anti-hSLPI antibody and FITC-labelled secondary antibody (empty peaks). Autofluorescence was given by control cells that were not incubated with the protein and are represented by peaks filled in red. Arbitrary fluorescent units (AFU) were quantified and are represented by the figure inset.

above 64 μg/mL and below 8 μg/mL define a strain as resistant or susceptible to this drug, respectively.[44] In this study, the evaluation of MIC showed distinct fluconazole values for the two strains of *C. albicans*, as expected. However, SLPI values were similar for both strains suggesting that the resistance mechanisms to fluconazole from the PRI strain were unable to affect SLPI activity. In fact, an MFS efflux pump was recently identified in the PRI strain as the main cause of its fluconazole resistance.[40] Therefore, similar MIC values for both susceptible and PRI strain makes us believe that this efflux mechanism may be ineffective against SLPI activity. Anyway, resistance to antifungal drugs has particular importance considering the limited number of commercially available drugs.[2] Thus, any new therapy able to circumvent this situation must be considered relevant.

SLPI MIC values for both strains were higher than 17 μM, which remains within the range of physiological secretion.[26] Therefore, considering its constitutive features, it is likely that an exogenous administration of SLPI could fulfil its antifungal biological role without any adverse effects on the patient.[45] The administration of 17 mM of recombinant SLPI, by aspiration, on the bronchopulmonary epithelium of patients with cystic fibrosis reduced proteolytic activity and the expression of interleukin 8 (IL-8) providing a significant improvement in the inflammation.[46] The inhibitory values obtained in this study, although high, seem promising and reinforce the idea of using SLPI as a possible therapeutic alternative.

Regarding growth and viability; the results showed that MIC and MFC values were similar, pointing primarily to a fungicidal activity. Clinically, the selection criteria for antimicrobial drugs are universal, and postulate that whenever there is an option, the chosen drug must be microbicidal in the first place,[47] reinforcing the potential of SLPI as a therapeutic option in the future.

The secreted protease activities of *C. albicans* have been associated with the increase in the virulence of this organism; therefore, the therapeutic use of protease inhibitors has been suggested as a potential alternative for the control and management of candidiasis.[11] The superfamily of subtilisin-like serine are reportedly responsible for activating protein precursors in *Saccharomyces cerevisiae* and *C. albicans*.[48] In these two genuses, a similar proenzyme prototype is Kex2p, which is a cytoplasmic serine protease involved in the expression of some pivotal virulence factors in *C. albicans*, including morphological differentiation and the clearance of extracellular proteases, which are factors considered essential to a successful infection.[12,49,50] In fact, this study demonstrated that the physiologic concentrations of SLPI were able to inhibit significantly the proteolytic activity of serine proteases secreted by *C. albicans*.

Adhesive interactions are considered the initial step that leads to the establishment of infections caused by any microorganism, including *Candida* spp.[51,52] The presence of SLPI during the interaction process between epithelial cells and *C. albicans* reduced it by more than 60%. Epithelial invasion

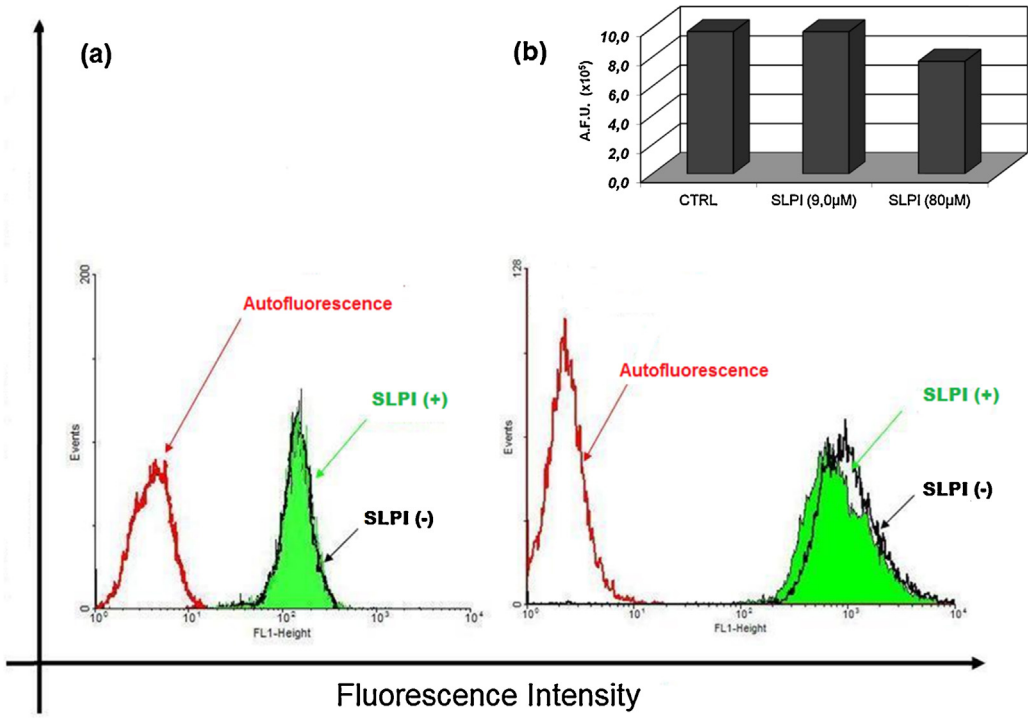

Fig. 3 – Histogram showing the binding of concanavalin A labelled with FITC to the surface of *C. albicans*, after treatment with 9.0 μM (a) or 80 μM (b) of SLPI for 6 h at 37 °C. Autofluorescence was given by not incubated nor labelled cells and is represented by red empty lines. Treated cells are represented by green filled peaks while untreated cells are expressed by solid empty black lines. Both treated and untreated cells were incubated with FITC labelled concanavalin A for 1 h and analyzed by flow cytometry. Arbitrary fluorescent units (AFU) were quantified, as well, and are represented by the figure inset.

by *C. albicans* is crucial to the pathogenesis of candidiasis and normally follows two different mechanisms: an active penetration, where *C. albicans* hyphae or germ tube elongates and pushes itself physically into the host cell,[53] and penetration by endocytosis, where adhesins bind to epithelial cell receptors and activate proper endocytosis machinery.[54] In fact, the role of serine proteinases in the adhesion process is considered crucial, as its inhibition by classical inhibitors,

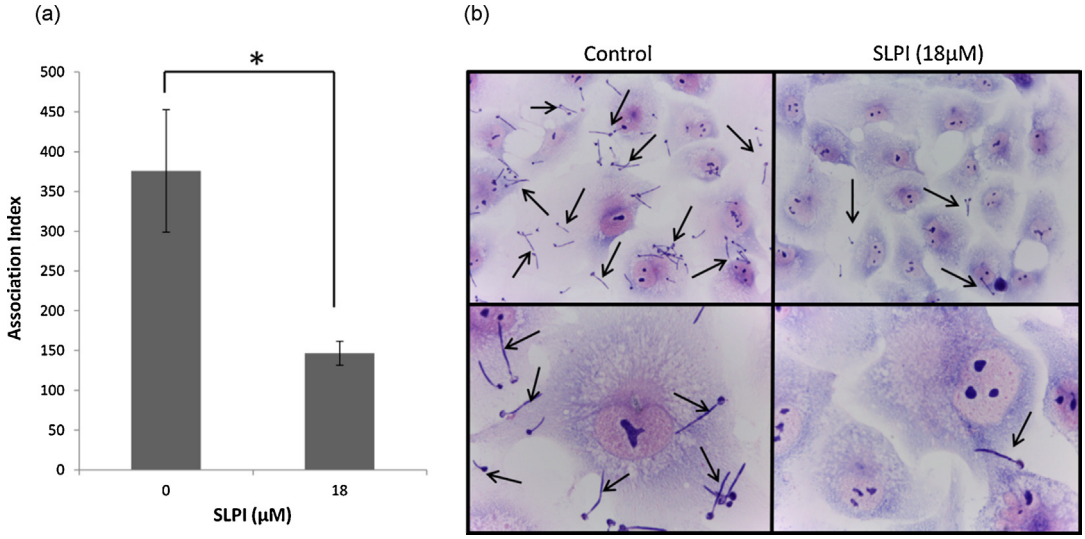

Fig. 4 – Effect of SLPI protein on the interaction between *C. albicans* and epithelial cells. The presence of 18 μM of SLPI during interaction reduced the adhesion process significantly (a). Representative images (b) are shown, and arrows point to *C. albicans* adhered to epithelial cells in both treated and control fields. Magnification 400× and 1000× (up and lower boxes, respectively). (*) $p = 0.003$.

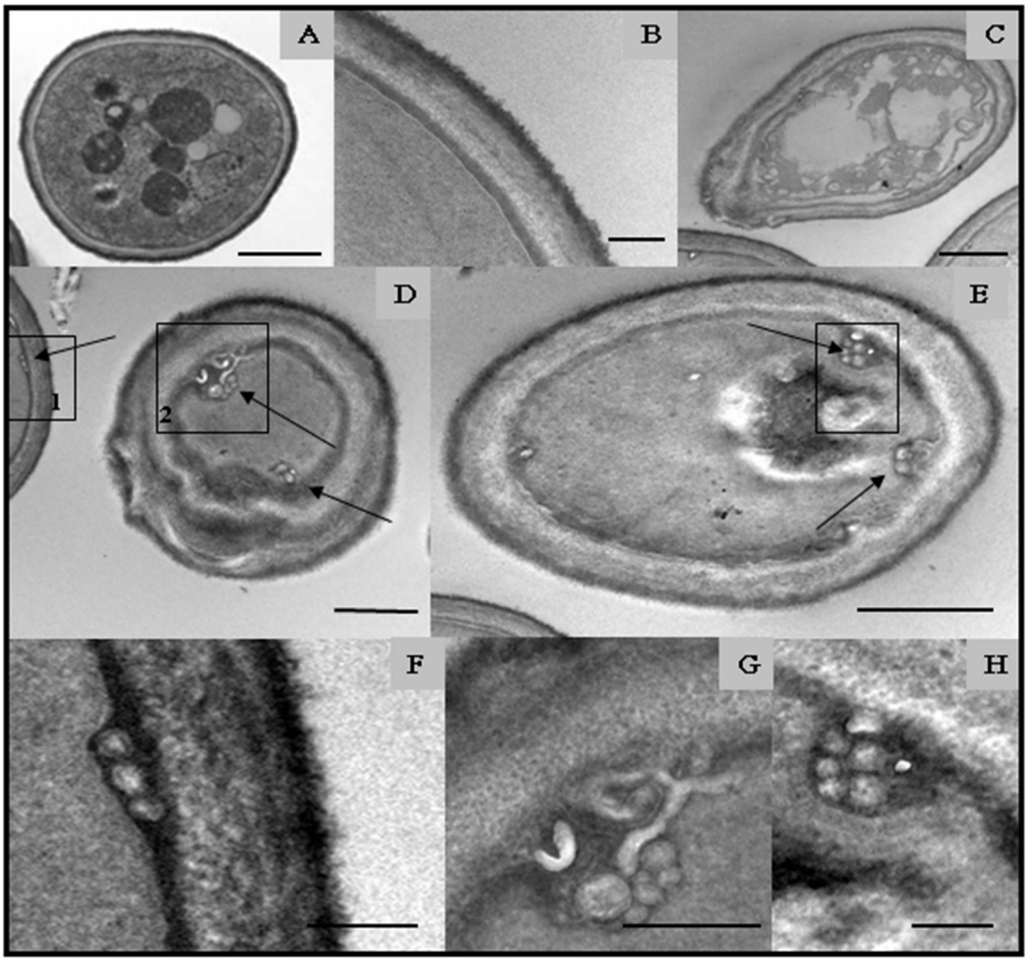

Fig. 5 – Transmission electron microscopy of *C. albicans* untreated cells (A and B) and cells treated with 80 μM of SLPI (C–H). Control cells show regular contour and electrondense cytoplasm (A) and a fungal cell wall with different layers electrondensities (B). Treated cells present hollow cytoplasm (C–E), and irregular membrane structures along the cytoplasmic membrane (arrows in D and E), The insets 1 and 2 in D correspond to F and G, respectively, and the inset in the E corresponds to H. Bars represent 0.2 μm (B, F, G and H) and 1.0 μm (A, C, D and E).

such as phenylmethylsulphonyl fluoride (PMSF) is able to downregulate association indexes to similar levels.[12] Histopathologically, the interaction process between fungal and epithelium can be considered a dynamic process that relies on proper features from the host, such as ageing or immunosuppression, and from the yeast such as hydrophobicity, positive net charge, presence of pathogen-associated molecular patterns (PAMP), and exposing of mannoproteins, among others.[55]

CSH has been considered a non biological factor strongly related to the adhesion of fungal cells to host tissues,[56–58] and to the yeast resistance to phagocytosis by polymorphonuclear cells.[59] This feature probably occurs due to the presence of hydrophobic proteins that can promote the binding to extracellular matrix components. But it is also possible that CSH could facilitate specific interactions between receptors and their ligants on the surface of the fungus.[60] In 2004, Hazen[59] described for the first time, a hydrophobic surface protein constitutive of *C. albicans* namely CSH1p, which is expressed independently of environmental conditions, culture or cell morphology. Recently and using a similar methodology, it was reported that sub-inhibitory concentrations of gluconate chlorhexidine were able to reduce *C. albicans* CSH in a dose dependent manner regardless of a patient's characteristics such as diabetic, asthmatic or smoker.[61] However, this study revealed an increase of 11.1% in CSH after treatment with 80 μM of SLPI. The results obtained by Ellepola and co-workers (2013)[61] could be explained by chlorhexidine pharmacodynamics characteristics, because this compound acts directly on the cell membrane as a cationic detergent, while SLPI may be evolved in other biological processes, such as proteinases inhibition.[19]

The surface of *C. albicans* has a variety of PAMPs, such as chitin, glucan, mannoproteins and glycolipids. These structures act as ligants for receptors that specifically recognize these patterns, normally present on the surface of the host cells. This host–fungal interaction exhibits features of a dynamic system, in which the same cell can easily switch between different morphological forms presenting different PAMPs at different moments of time, thereby modulating the

survival and virulence of commensal fungi such as *C. albicans*.[62–64] *C. albicans* has the ability to mask certain cell wall components and unmask others, qualitatively changing the PAMPs that are exposed to the host. For instance, dectin-1 recognizes β-glucans on fungal cell walls and triggers immune responses by the production of pro and anti-inflammatory cytokines. However, while the yeast form displays β-glucans on its surface, the hyphal form shields its layer of β-glucans underneath a coat of mannoproteins.[65] On the assumption that *C. albicans* expresses molecules capable of binding to SLPI, flow cytometry analysis was carried out. The results showed that the cells were strongly recognized by the anti-hSLPI, in a dose-dependent manner, suggesting the existence of binding sites for this protein on the surface of *C. albicans*, which can contribute to the construction of alternative models to manage infections caused by this organism by blocking these sites.

Cell wall glycoconjugates have an important role in the interaction between fungus and host cells.[66] To evaluated the effect of SLPI on the expression of cell surface mannoproteins of *C. albicans*, this study used FITC labelled Concanavalin A due to its ability to bind to mannose residues. The results showed that treatment with 9.0 μM of SLPI was not enough to change the availability of mannoproteins on the cell wall. However treatment with the maximal physiologic concentration (80 μM) reduced the binding of concanavalin A to the cell surface by 20%, pointing to a possible reduction in the expression of cell wall mannoproteins or a decreasing in mannose residues available for the connection to the lectin. In fact, con-A has been employed as a viable marker to evaluate the expression of mannans and mannoproteins on the surface of microorganisms.[67] These structures have a key role not only in maintaining the structural integrity of the cell, but also in the process of adhesion to host cells.[68,69] Thus, the possible reduction in the expression of mannoproteins in *C. albicans*, as suggested by treatment with 80 μM of SLPI indicates an important potential on modulating one virulence factor that can impair yeast adhesion to the epithelium. As previously stated, fungal cell walls are complex, dynamic and highly adaptable organelle. *C. albicans* alters the phenotype of its wall by changing mannoproteins and polymer distribution in response to environmental conditions. Hence, some authors have already speculated that the variation in fungal cell wall phenotypes may have important implications for host–pathogen interactions.[70]

The effect of SLPI on the ultrastructure of *C. albicans* was evaluated by TEM. Yeasts treated with 80 μM of SLPI revealed cytoplasmatic deflation, and the presence of disorganized plasmatic membrane like structures on the inner side of the plasma membrane within the cytoplasm compartment. This result suggests that the SLPI protein was able to overcome the cell wall and interact with the cytoplasmic membrane or at least interfere with the synthesis of its components. In fact, a previous study has already reported structural changes in the plasmatic membrane after the treatment of *Cryptococcus neoformans* with inhibitors of the synthesis of ergosterol[71] similar to this study.

In summary, the results obtained in this study point to the possible application of SLPI as a viable alternative in the treatment and management of infections caused by *C. albicans*, since this inhibitor was able to exert an important influence on the viability and on relevant virulence mechanisms of *C. albicans*. Considering this, the exogenous administration of this protein could perhaps become a viable therapeutic alternative in the future, even for *C. albicans* resistant strains, overcoming toxicity issues, considering its constitutive and physiological features.

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Competing interests

None declared.

Ethical approval

Not required.

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